NTP
National Toxicology Program

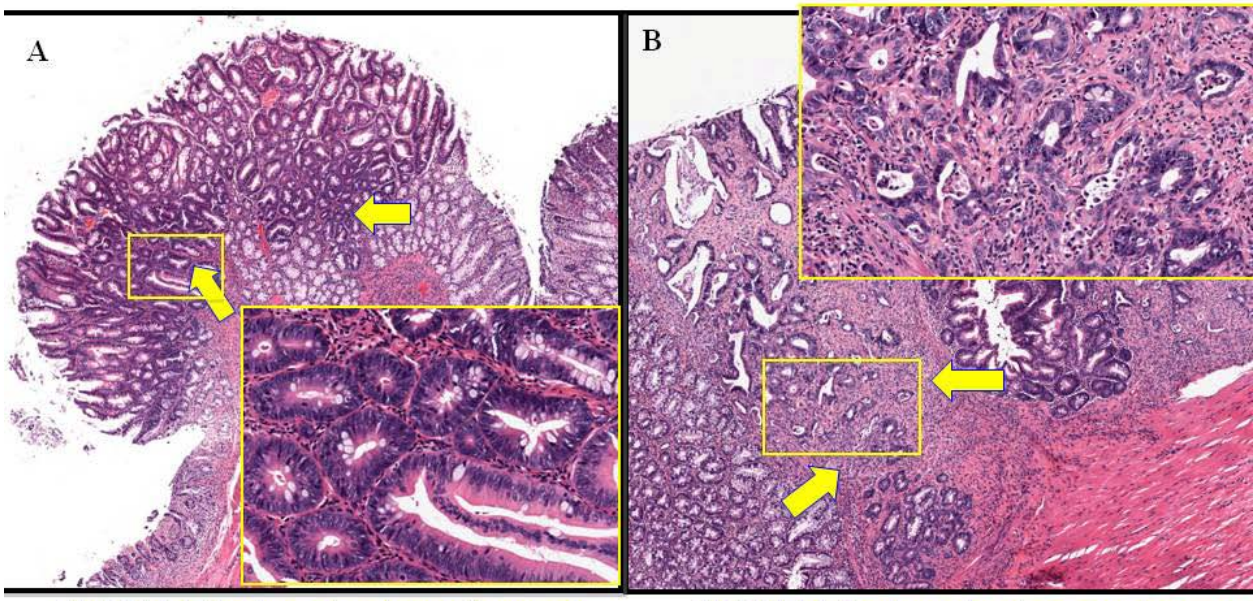
Aloe vera non-decolorized whole leaf extract (AVNWLE)-induced large intestinal tumors in F344 rats exhibit similarities with human sporadic colon cancer

Arun Pandiri, BVSc&AH, MS, PhD, DACVP
National Institute of Environmental Health Sciences
(present address: Experimental Pathology Laboratories, Inc., Research Triangle Park, NC)

Technical Reports Peer Review Meeting
April 5, 2011

NTP
National Toxicology Program

AVNWLE-induced large intestinal adenomas and carcinomas in F344 rats have morphological features similar to human colon cancer



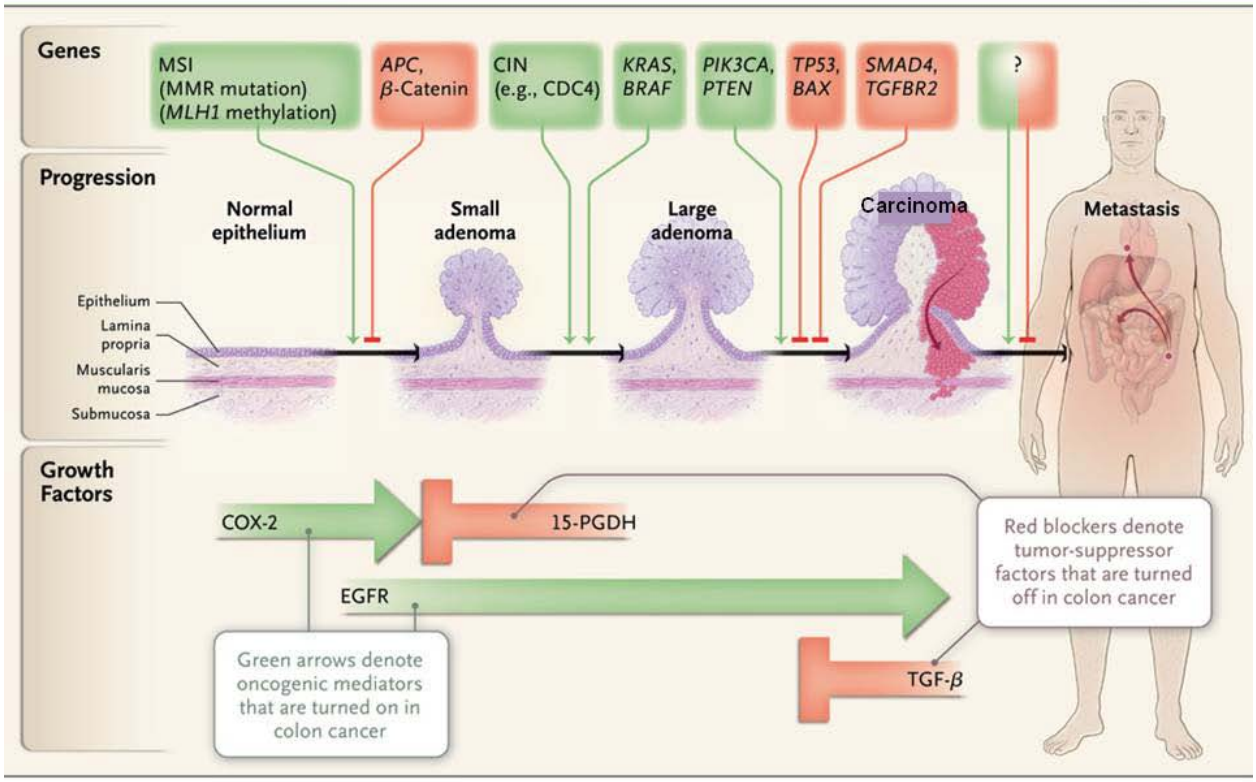
AVNWLE-induced colon adenoma

AVNWLE-induced colon carcinoma

Colon Cancer in Humans

- 4th commonly diagnosed cancer, but 2nd leading cause of cancer-related death
- Based on genetic origin - 15%
  - Familial adenomatous polyposis (FAP)
  - Hereditary nonpolyposis colon cancer (HNPCC) aka Lynch syndrome
- Sporadic CRC – 85%

NTP
National Toxicology Program



Markowitz and Bertagnolli, N Engl J Med 2009;361:2449-60.

Hypothesis:

Genetic alterations within AVNWLE-induced large intestinal tumors in F344 rats are similar to sporadic colon cancer in humans.

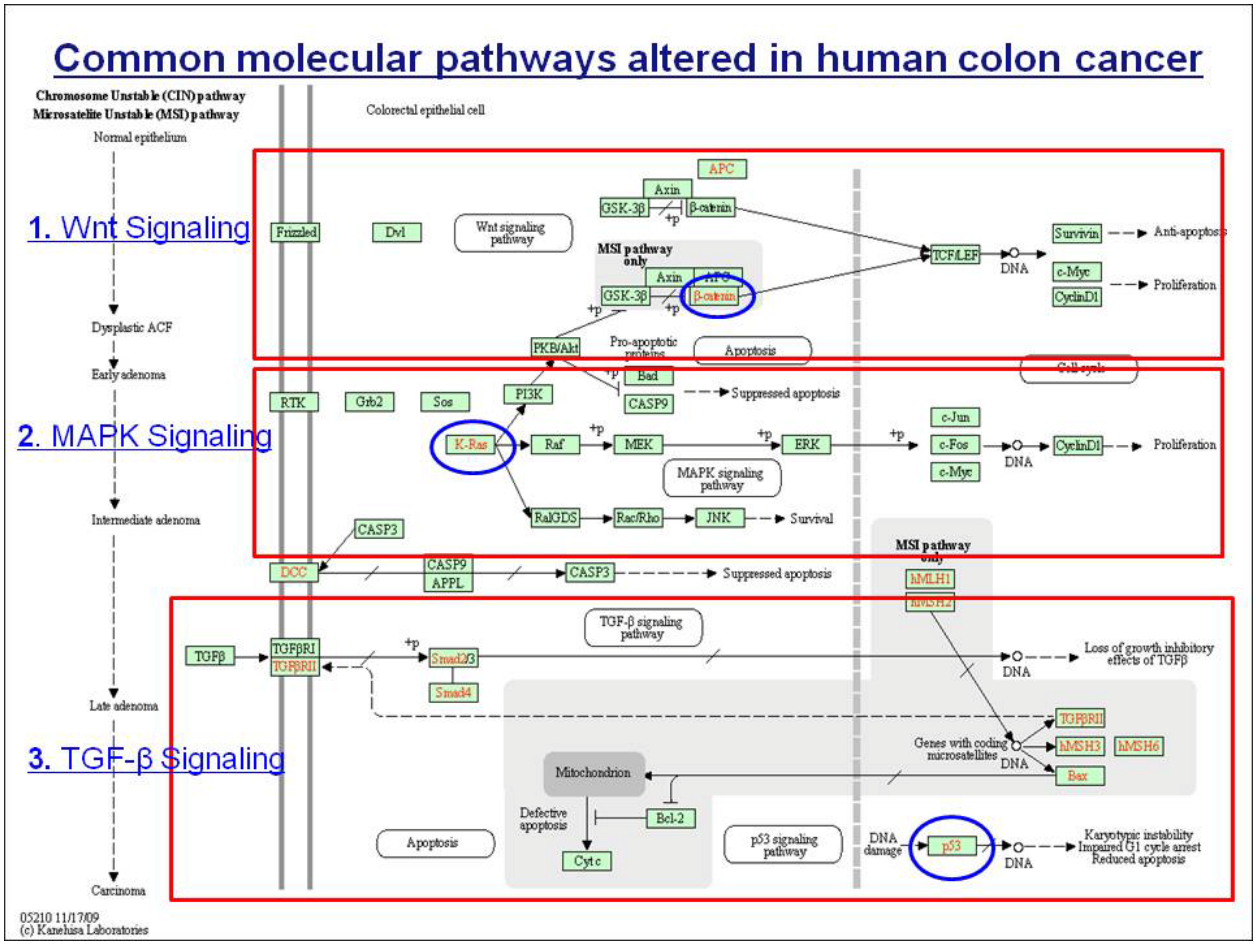
Common genetic alterations in human and rodent colon tumors

Species	Carcinogen	Lesion	Mutation frequency (%)				
			APC	*β-Catenin*	*K-ras*	*DCC*	*p53*
Human		Adenocarcinoma	40–80	15	40–60	40–70	50–80
		Adenoma	40–65	—	0–40	—	—
		ACF	<5	—	10–95	—	—
Rat	AOM	Adenocarcinoma	8	75	30–60	—	0
		ACF	0	—	20–40	—	0
	PhIP	Adenocarcinoma	13	50	0	—	0
	MNU	Adenocarcinoma	—	—	18	—	27
Mouse	AOM	Adenocarcinoma	—	100	0–10	—	0

Takahashi and Wakabayashi, Cancer Sci (2004) 95:6, 475–480

"–" not tested

Common molecular pathways altered in human colon cancer



Experimental design

1) Mutation analysis

- DNA from adenoma (8), carcinoma (4), and untreated control colons (4)
- "Hot spots" for mutations relevant to human colon cancer
  - *Ctnnb1* (β-catenin) (exon 2)
  - *Kras* (exons 1-2)
  - *Tp53* (exons 5-8)

2) Pathway analysis

- RNA from adenoma (4), carcinoma (4), and untreated colonic epithelium (4)
- Evaluation of pathways involved in human colon cancer
  - WNT pathway (84 genes) - Catalogued PCR array
  - MAPK pathway (84 genes) - Catalogued PCR array
  - TGF-β pathway (32 genes)
  - Other colon cancer genes (60 genes)

} Custom-designed PCR array

Results

Mutation frequency in AVNWLE-induced large intestinal tumors

Group	*Ctnnb1* mutations (Codons 32, 34, 41, 45)	*Kras* mutations (Codons 12, 13, 61)	*Tp53* mutations (Exons 5-8)
Untreated Colon	0/4	0/4	0/4
Adenoma	3/8	2/8	0/8
Carcinoma	1/4	2/4	0/4

Group	% *Ctnnb1* mutations	% *Kras* mutations	% *Tp53* mutations
AVNWLE	33	33	0
Human CRC	15-26	40-60	50*
Azoxymethane	50-80	30-60	0
Heterocyclic Amines	50-75	0-14	0

*p<0.05

**Kras* and *Ctnnb1* mutation frequency is similar to that of sporadic human colon cancer

Results

Mutation frequency in AVNWLE-induced large intestinal tumors

Group	*Ctnnb1* mutations (Codons 32, 34, 41, 45)	*Kras* mutations (Codons 12, 13, 61)	*Tp53* mutations (Exons 5-8)
Untreated Colon	0/4	0/4	0/4
Adenoma	3/8	2/8	0/8
Carcinoma	1/4	2/4	0/4

Group	% *Ctnnb1* mutations	% *Kras* mutations	% *Tp53* mutations
AVNWLE	33	33	0
Human CRC	15-26	40-60	50*
Azoxymethane	50-80	30-60	0
Heterocyclic Amines	50-75	0-14	0

*p<0.05

**Kras* and *Ctnnb1* mutation frequency is similar to that of sporadic human colon cancer

Wnt signaling in AVNWLE-induced large intestinal tumors

39/84 genes relevant to human colon cancer significantly altered

1) β-catenin-dependent Wnt signaling
   - APC complex, Wnt ligand binding
2) β-catenin-independent Wnt signaling pathways
   - Wnt/Ca^{2+} pathway
   - Wnt/planar cell polarity (PCP) pathway

Mediators of Wnt signaling	Genes
Wnt ligands	*Wif1, Wnt3, Wnt4, Wnt7b*
APC complex	*Axin1, Dvl1, Frzb, Fzd2, Fzd5, Fzd6*
Other genes	*Ctnnb1, RhoA, Bcl9, Dkk3, Nkd2, Sfrp1, Sfrp4*
Non-canonical Wnt signaling	*Wnt5a, Fzd6, Dvl1, RhoA, Nkd1, Nkd2*

Wnt signaling in AVNWLE-induced large intestinal tumors

39/84 genes relevant to human colon cancer significantly altered

1) β-catenin-dependent Wnt signaling
   - APC complex, Wnt ligand binding
2) β-catenin-independent Wnt signaling pathways
   - Wnt/Ca^{2+} pathway
   - Wnt/planar cell polarity (PCP) pathway

Mediators of Wnt signaling	Genes
Wnt ligands	*Wif1, Wnt3, Wnt4, Wnt7b*
APC complex	*Axin1, Dvl1, Frzb, Fzd2, Fzd5, Fzd6*
Other genes	*Ctnnb1, RhoA, Bcl9, Dkk3, Nkd2, Sfrp1, Sfrp4*
Non-canonical Wnt signaling	*Wnt5a, Fzd6, Dvl1, RhoA, Nkd1, Nkd2*

TGF-β signaling in AVNWLE-induced large intestinal tumors

48/92 genes relevant to human colon cancer significantly altered

Mediators of TGF-β signaling	Genes
Transcription regulators	*Smad1, Smad2, Smad5*
Growth factors	*Tgfb1, Tgfb2, Tgfb3, Bmp1, Bmp4, Inhba*
Kinases	*Tgfbr1, Tgfbr2, Tgfbr3*

Other important genes in Colon cancer	Genes
Transcription regulators	*Klf4, Sox9, Sox4, Stat1, Stat3, Tcf7l2*
Kinases	*Pik3cb, Pik3r1, Pik3r1,Akt1, Akt2, Akt3, Fgfr1, Sgk1, Stk11*
Phosphatases	*Cdc25a, Dusp4, Ptpro Ptprs*
Apoptosis	*Birc5, Bax, Casp3*
Other significant colon cancer genes	*Tnf-α, Nos2, Ca2, Hpgd, Hsd17b2, Msh2, Psat1, Timp1, Hspd1, Top2a, Sparc*

Conclusion

- AVNWLE-induced colon tumors in F344 rats
  - Contain point mutations in *Kras* or *Ctnnb1*
  - Appear not to have *Tp53* mutations
  - Have alterations within Wnt, MAPK, and TGF-β signaling pathways
- AVNWLE-induced colon tumors in F344 rats share morphological and molecular features with human colon cancer

Acknowledgements

National Toxicology Program

Dr. Robert Sills
Dr. Mark Hoenerhoff
Dr. Shyamal Peddada
Mr. Thai-Vu Ton
Ms. Hue-Hua Hong
Dr. Gordon Flake
Dr. David Malarkey
Dr. Nigel Walker

National Center for Toxicological Research

Dr. Mary Boudreau
Dr. Igor Pogribny

Future Directions

Collaboration with NCTR

- Compare genetic, epigenetic and protein changes in tumors and histologically normal colon tissue adjacent to tumors
- Subchronic studies of Aloe vera and Senna
  - Detect early genetic and epigenetic alterations within colonic mucosa
  - Study the progression of early lesions: histologically normal colon> aberrant crypt foci & mucin depleted foci > adenoma

NTP
National Toxicology Program

	Tumor suppressor activities	Pro-oncogenic activities
Initiated cell target	Growth Inhibition Apoptosis Negative angiogenic regulator profile Maintenance of genomic stability Induction of replicative senescence Prevention of immortalization Maintenance of tissue architecture	Enhanced epithelial → mesenchymal transition Increased motility Increased invasiveness Increased colonization of bone (PTHrP secretion) Growth stimulation
Stromal target	Maintenance of tissue architecture?	Suppression of immune surveillance Increased angiogenesis

Normal epithelium

Suppressor activities dominate

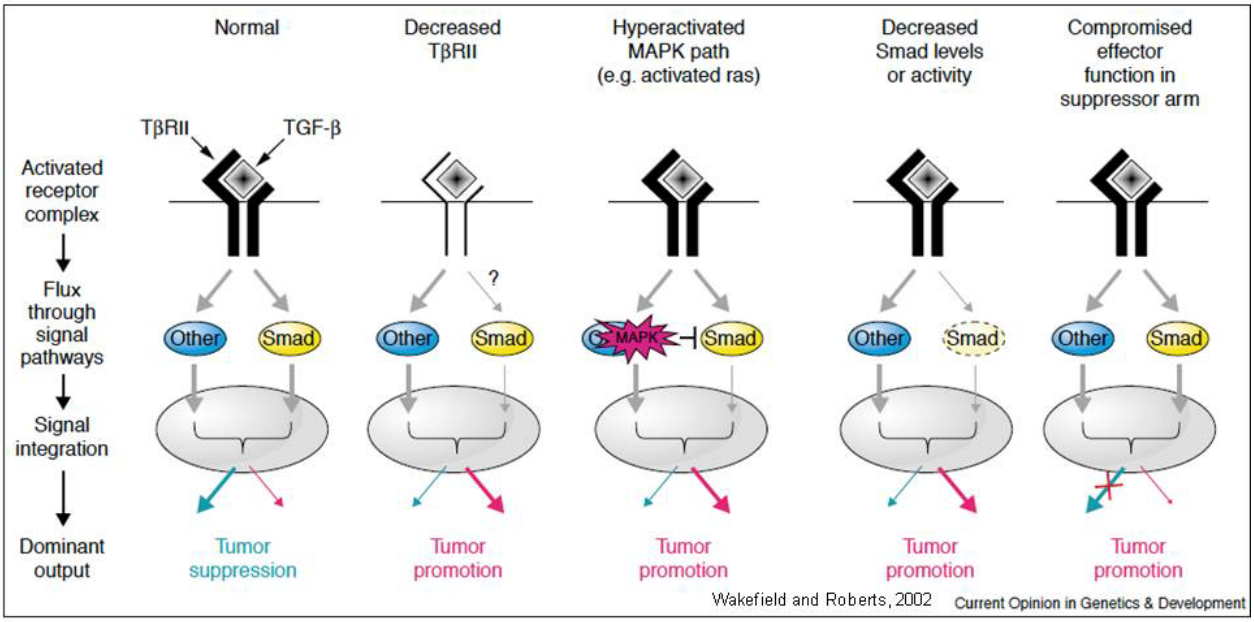
Reduced or altered epithelial responsiveness to TGF-βs

Increased TGF-β production or activation

Invasive metastatic cancer

Oncogenic activities dominate

Current Opinion in Genetics & Development Wakefield and Roberts, 2002


Normal
Decreased TβRII
Hyperactivated MAPK path (e.g. activated ras)
Decreased Smad levels or activity
Compromised effector function in suppressor arm
TβRII
TGF-β
Activated receptor complex
Flux through signal pathways
Signal integration
Dominant output
Other
Smad
?
MAPK
Tumor suppression
Tumor promotion
Tumor promotion
Tumor promotion
Tumor promotion
Wakefield and Roberts, 2002
Current Opinion in Genetics & Development

NTP
National Toxicology Program

Summary of Mutation Analysis

Sample No.	Dose (%)	Animal ID	Dx	K-*ras* Cdn 12	K-*ras* Cdn 13	K-*ras* Cdn 61	β-Catenin	*p-53* Exon 5-8	
6	1.0	1712	Ad	NM	GGC->CGC Gly->Arg	NM	NM	NM	NM
7	1.0	1711	Ad	NM	NM	NM	**Codon 32** GAT->AAT Asp->Asn	NM	NM
8	1.0	1812	Ad	NM	NM	NM	NM	NM	NM
9	1.5	1301	Ad	NM	NM	NM	**Codon 45** TCC->TTC Ser->Phe	NM	NM
10	1.5	1102	Ad	NM	NM	NM	NM	NM	NM
11	1.5	1602	Ad	NM	GGC->CGC Gly->Arg	NM	NM	NM	NM
12	1.5	1922	Ad	NM	NM	NM	**Codon 41** ACC->CCC Thr->Pro	NM	NM
13	1.0	1052	Ca	NM	NM	CAA->CGA Glu->Arg	NM	NM	NM
14	1.0	1151	Ca	GGT->GAT Gly->Asp	NM	NM	NM	NM	NM
15	1.0	1402	Ca	NM	NM	NM	**Codon 34** GGA->GAA Gly->Glu	NM	NM
16	1.5	1921	Ca	NM	NM	NM	NM	NM	NM